blood specimens plus several other tests on heated serum are presented.

2. The relative reproducibility of the Chediak and Chediak-VDRL tests among the five participating laboratories is shown in tabular form and is discussed.

3. Relative efficiency of the tests on dried blood specimens, as compared to tests on heated serum as "detector" tests for syphilis is discussed.

4. The micro-VDRL slide test findings, as reported by two laboratories, are presented and compared with results of other testing procedures.


#### REFERENCES

(*1*) Harris, A., Olansky, S.: A study of the filter paper microscopic (FPM) test for syphilis. Preliminary report. J. Ven. Dis. Inform. 32: 1–4 (1951).

(*2*) Harris, Ad, Vinson, Hulda, Olansky, Sidney: A study of the Chediak test. Pub. Health Rep. 67: 572–576 (1951).

(*3*) Cannefax, G. R., Beyer, H. R., and Johnwick, E. B.: A micromodification of the VDRL slide test. Pub. Health Rep. 67: 576–581 (1952).

(*4*) U. S. Public Health Service: Manual of serologic tests for syphilis. Supplement No. 22, J. Ven. Dis. Inform., 1949, p. 110; (*a*) Ibid., p. 68; (*b*) Ibid., p. 69; (*c*) Ibid., pp. 38–39.

(*5*) Mazzini, L. Y.: Mazzini cardiolipin microflocculation test for syphilis. J. Immunol. 66: 261–275 (1951).


# The Chediak Test— A Preliminary Report


**By AD HARRIS**
**SIDNEY OLANSKY, M.D.**
**HULDA VINSON, B.S.**


The development of a test for syphilis requiring only a small amount of blood that could be collected with a minimum of equipment and difficulty by relatively untrained personnel has been the object of several investigative studies (*1–9*). Such a test would aid considerably in the detection of cases of syphilis from which collection of the amounts of blood necessary for the standard testing procedures, using serum, is difficult or impractical due to lack of either adequate facilities or adequately trained workers.

In 1932 Dr. Alejandro Chediak of Havana, Cuba, published a technique for the serodiagnosis of syphilis requiring the collection of only a single drop of blood. The Venereal Disease Research Laboratory has recently studied this method as it was demonstrated by Dr. Chediak and explained in a personal communication from him. The purpose of this presentation is to report results obtained with the Chediak test and modifications of this technique using cardiolipin-lecithin antigens, under specified conditions.

## CHEDIAK TEST

The mechanics of the Chediak test were retained with only minor changes throughout this study, using equipment and antigen supplied by Dr. Chediak. A brief summary of this method as demonstrated by Dr. Chediak during a visit to the Venereal Disease Research Laboratory, follows:

1. A drop of dried, "homogenized" blood, collected on a glass slide, is resuspended in 0.03 ml. of 3.5-percent sodium chloride solution. This is accomplished by placing the slide in a slide holder that forms a well above the blood sample so that two ¼-inch steel balls may be put into each blood-saline mixture. The blood is then dissolved or resuspended by rotating the slide holder for approximately 1 minute.

2. After 0.03 ml. of antigen emulsion is added to each specimen, the specimens are rerotated on a flat-bed rotator for 3 minutes at 180 rpm.

3. Steel balls are removed, glass covers are placed into slide holders to prevent drying, and specimens are allowed to stand 20 to 30 minutes before being examined.

4. Slide holder covers are removed and specimens are read with a microscope at 60× maginfication. Small clumps of antigen particles are interpreted as a doubtful reaction, large clumps indicate a positive reaction, and no clumping of antigen particles is read as a negative reaction.

---


*Mr. Harris is assistant director of the Venereal Disease Research Laboratory, Venereal Disease Division, Public Health Service, Chamblee, Ga., and is in charge of the serology section; Miss Vinson is a bacteriologist in the research unit of the serology section; Dr. Olansky is director of the Laboratory.*


---

The original Chediak antigen used in this test is a cholesterolized alcoholic extract of beef heart to which Tolu and Peru balsams have been added.

### MODIFIED CHEDIAK TECHNIQUE (CHEDIAK-VDRL TEST)

I. Reagents:
1. VDRL flocculation antigen (*10*).
2. VDRL buffered saline solution (*10*).
3. 3.5-percent sodium chloride solution (prepared by dissolving 3.5 gm. dry sodium chloride in 100 ml. freshly distilled water).

II. Equipment:
*1. Chediak 3-piece holders.
*2. ¼-inch steel ball bearings.
*3. Electromagnet or forceps.

III. Preparation of Antigen Emulsion:
1. Prepare and check VDRL antigen emulsion as directed on page 110 of the Manual of Serologic Tests for Syphilis, Supplement 22 to the Journal of Venereal Disease Information.
2. Prepare a diluted VDRL antigen emulsion by adding 1 part of VDRL buffered saline solution to 1 part of VDRL antigen emulsion. This diluted emulsion should be allowed to stand 10 minutes before use and should be used within 1 hour.

IV. Technique:
*1. Place slides on holder, fastening top to make a well around each specimen.
*2. Add two ¼-inch ball bearings to each specimen.
*3. Add 0.03 ml. of 3.5-percent sodium chloride solution to each specimen. This may be accomplished by delivering the solution from a 0.2-ml. pipette (graduated in 0.01 ml.) or by dropping from a syringe fitted with a 15-gauge needle held in a vertical position. The needle should be tested for delivery of 0.03 ml. of 3.5-percent sodium chloride solution on the day of use.
*4. Shake slide holders with an irregular motion for 1 minute or until dried blood is resuspended.
5. Add to each specimen 0.03 ml. of diluted VDRL antigen emulsion from a 0.2-ml. pipette graduated in 0.01 ml.
*6. Rotate at 180 rpm for 3 minutes.
*7. Remove ball bearings with electromagnet or forceps.
8. Read tests immediately, using microscope with 60× magnification.
9. Report as follows:

| | |
|---|---|
| Reactive | Definite clumping of antigen particles. |
| Nonreactive | No clumping of antigen particles. |

NOTE: All items marked with an asterisk are identical with those in the Chediak test.

## Collection of Blood Specimens

Dried blood specimens were collected on 3- x 1-inch glass slides with frosted ends. An identifying number was written in pencil on the frosted portion of the slide. In this way the blood sample and identification were never separated from time of collection until testing was completed.

Slides were ringed on the reverse side with a wax pencil so that the dried blood specimen would coincide with the well formed when the slide was placed in the plastic slide holder. A drop of blood, obtained by puncturing a finger, toe, or heel with an automatic spring-type lancet, was allowed to fall onto the ringed portion of a properly labeled slide. The blood was then "homogenized" by stirring with an applicator stick for ½ to 1 minute.

Multiple slides of dried blood specimens and venipuncture blood samples for serum tests were collected simultaneously for comparative testing.

## Procedure

In the first series of specimens tested with a cardiolipin-lecithin antigen an attempt was made to keep the reagin-antigen-particle ratio approximately the same as in the VDRL slide test. Since the dried blood was resuspended in 0.03 ml. saline (approximately one-half the 0.05 ml. serum of the standard test) the dose of antigen was reduced to a drop equivalent to $\frac{1}{120}$ ml. Rapid drying of these preparations during the rotation period rendered them unsatisfactory. To increase the amount of fluid and maintain the same reagin-antigen-particle ratio, the VDRL antigen emulsion was diluted in the proportion of 1 part of antigen emulsion to 3 parts of VDRL buffered saline solution and the dose of this diluted emulsion was set at 0.03 ml. With this combination, results approximately equal in sensitivity to those produced by Chediak antigen were obtained.

A second series of tests using 0.03 ml. of antigen emulsion, containing equal parts of VDRL antigen emulsion and buffered saline solution, gave fewer negative reactions in specimens from syphilitic donors than did other antigen-saline combinations tested. For this reason, this type of antigen emulsion was selected for the Chediak-VDRL test. Another advantage of the diluted VDRL antigen over the Chediak antigen, in the Chediak test, is that reactions were more rapid so that, when the VDRL antigen was used, the 20-minute waiting period of the Chediak test could be discarded, and reactions

could be read immediately after the 3-minute rotation period.

Attempts to further increase sensitivity by increasing the concentration of the sodium chloride solution used for resuspension of the dried blood resulted in crystallization of sodium chloride, which interfered with readings. Prolongation of the rotation time increased sensitivity but also produced very rough negative and weakly positive reactions on known negative donors. Several cardiolipin-lecithin antigens other than the one for the VDRL slide test were employed in varying dilutions without obtaining an increase in test sensitivity.

In order to determine the effect of storage on dried blood samples, multiple specimens were collected from unselected patients undergoing treatment for syphilis. These specimens were stored at room temperature for varying periods of time before being tested. Results obtained with the Chediak and Chediak-VDRL tests on dried blood specimens stored for 24 and 72 hours are listed in table 1.

Duplicate dried blood specimens and whole-blood specimens in vacutainers were simultaneously collected from a selected group of patients. These specimens were used to determine the relative capacities of the two tests on dried blood to detect those donors whose serum produced positive or weakly positive reactions in the VDRL slide test. Results obtained in this comparison are recorded in table 2.

**Table 1. Effect of storage at room temperature on reactivity of dried blood specimens from 67 donors**

| Reactivity after 72 hours' storage | Reactivity after 24 hours' storage | | | |
|---|---|---|---|---|
| | Chediak test | | | |
| | Positive | Doubtful | Negative | Total |
| Positive | 5 | 1 | 0 | 6 |
| Doubtful | 8 | 6 | 5 | 19 |
| Negative | 6 | 11 | 25 | 42 |
| Total | 19 | 18 | 30 | 67 |
| | Chediak-VDRL test | | | |
| | Reactive | Nonreactive | Totals | |
| Reactive | 47 | 1 | 48 | |
| Nonreactive | 5 | 14 | 19 | |
| Total | 52 | 15 | 67 | |

### Effect of Storage on Dried Blood Specimens

Several dried blood samples were collected from each of a group of patients under treatment for syphilis in order to determine the effect of storage on this type of blood sample. These specimens were tested after storage at room temperature for 24 hours, 72 hours, and longer periods.

Some deterioration in reactivity was noted at all storage periods greater than 24 hours, and the longer storage periods produced the greatest loss in reactivity. However, since 72 hours was the shortest time that could be used for interlaboratory studies involving shipment of specimens to distant parts of this country and since this period would also be considered maximum for studies conducted by a single laboratory with state-wide blood collections submitted by mail, attention was specifically directed to the effect of this much delay between collection and testing on dried blood specimens.

Results presented in table 1 show that fewer "negative" reactions were obtained by both techniques at the earlier testing period and that a greater loss of reactivity was noted in Chediak test results on specimens stored for 72 hours than in the findings of the Chediak-VDRL test. The number of specimens used in this series is too small to indicate definite sensitivity positions of the tests used but they do serve as an indicator of relative test behaviors.

Storage conditions were not unusually humid during this study. To ascertain the effects of humidity on the dried blood samples during a storage period, several collections were placed in a glass jar over water, at room temperature. Under these conditions the blood specimens stored for 24 or more hours were unsatisfactory for testing due to presence of gross debris that did not redissolve in the saline.

### Comparison of Results With Two Techniques

A series of specimens from 196 donors was tested, using the Chediak and Chediak-VDRL

techniques. Blood specimens collected by venipuncture at the same time were tested, using the VDRL slide test. The results obtained are presented in table 2.

One blood specimen that gave a negative reaction in the VDRL slide test had a positive Kahn test (32 units) and a 1 plus reaction in the Kolmer test. This specimen was positive in the Chediak test and reactive in the Chediak-VDRL test. The nine specimens that gave negative reactions in the VDRL slide test were also negative with both the Kahn and Kolmer tests. The remainder of these serums that gave positive reactions in the VDRL slide test also gave doubtful or positive reactions in either or both the Kahn and Kolmer tests.

Although the Chediak test gave positive or doubtful reactions on a few specimens that were reported nonreactive by the Chediak-VDRL procedure, 88 negative reactions were reported by the Chediak method and only 47 nonreactive results were obtained with the Chediak-VDRL test. The greatest discrepancy, in this regard, was found in the specimens of higher titer.

The highest percentage failure of the Chediak-VDRL to detect specimens that reacted in the VDRL slide test existed in those having 4 dils or less reactivity. In this zone, 39 of 65 VDRL slide test reactors were detected and in the group having more than 4 dils reactivity 109 of 121 reactors were found to be reactive by the Chediak-VDRL method.

## Discussion

The Chediak and Chediak-VDRL tests were both found to be less sensitive or reactive than the VDRL slide test on a selected group of donors. Results obtained with dried blood specimens stored for varying periods of time before being tested indicate that loss of reactivity will accompany delay in testing this type of blood specimen. These two factors militate against the use of these tests on dried blood in preference to the more reactive tests for syphilis performed on heated serum.

The principal recommendations for tests for syphilis performed on dried blood are: (*a*) 100-percent collection of specimens may be expected even from infants since only one drop of blood is required; (*b*) blood may be collected by puncture of finger, toe, or heel with a minimum of apparatus; and (*c*) collection may be made by relatively untrained personnel. The tests must, however, be performed with standardized reagents by adequately trained laboratory personnel.

The balance of these factors favors the tests for syphilis that are performed on serum rather than on whole blood if the detection of syphilis is of paramount interest. The Chediak-type tests will, however, have a definite place under circumstances that will not allow the collection of larger quantities of blood.

The findings presented in this article are not

**Table 2. Relative reactivity of Chediak, Chediak-VDRL, and quantitative VDRL slide tests on specimens from 196 patients**

| Test | Quantitative VDRL slide test | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Negative | 4 dils or less | | | | Over 4 dils | | | | | | | |
| | | <1 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | Total |
| Chediak: | | | | | | | | | | | | | |
| Positive | 1 | 0 | 3 | 7 | 8 | 8 | 13 | 10 | 4 | 5 | 0 | 2 | 61 |
| Doubtful | 3 | 2 | 2 | 6 | 6 | 7 | 3 | 7 | 6 | 3 | 1 | 1 | 47 |
| Negative | 6 | 5 | 6 | 6 | 14 | 16 | 8 | 7 | 9 | 6 | 1 | 4 | 88 |
| Total | 10 | 7 | 11 | 19 | 28 | 31 | 24 | 24 | 19 | 14 | 2 | 7 | 196 |
| Chediak-VDRL: | | | | | | | | | | | | | |
| Reactive | 1 | 2 | 5 | 9 | 23 | 30 | 22 | 23 | 17 | 12 | 0 | 5 | 149 |
| Nonreactive | 9 | 5 | 6 | 10 | 5 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 47 |
| Total | 10 | 7 | 11 | 19 | 28 | 31 | 24 | 24 | 19 | 14 | 2 | 7 | 196 |



203544—52——6

comprehensive enough to delineate clearly the relative efficiencies of the tests examined and are presented only as a preliminary report. Further study of the Chediak tests will be the subject of a later report.

NOTE: Since this study was completed, further trials have indicated that the reactivity coverage of the Chediak-VDRL test may be expanded by using both a diluted and an undiluted antigen emulsion.


## Summary

1. Results obtained with the Chediak and Chediak-VDRL tests on dried blood specimens stored for 24 and 72 hours at room temperature are presented.

2. The results of the Chediak, Chediak-VDRL, and quantitative VDRL slide tests on blood specimens from 196 donors are presented.

3. A modified Chediak test technique (Chediak-VDRL) is described.

4. Advantages and disadvantages of Chediak-type tests are discussed.


### REFERENCES


(1) Chediak, A.: El diagnostico de la sifilis practicado en una gota de sangre desecada y desfibrinada. Arch. med. inf. 1: 3-14 (1932).

(2) Dahr, P.: Die Luesdiagnose aus einem angetrockneten Tropfen Blut. Deutsche med Wchnschr. 60: 94-96 (1934).

(3) Guo, K. D.: Ein Fliesspapierverfahren zur Flockungsreaktion der Syphilis. I. Mitteilung. Deutsche med. Wchnschr. 64: 675-677 (1938).

(4) Vogt, H.: Untersuchungen über die Chediaksche Trockenblutreaktion und eine Abanderung der dahrschen Technik. Med. Welt. 12: 1802-1804 (1938).

(5) Meinicke, E., und Fischer, R.: Die Trockenblut-MKR. II Klin. Wchnschr. 18: 1060-1062 (1939).

(6) Zimmermann, E.: Die Chediak—M.K.R. II. Untersuchungen über neue Moglichkeiten. Med. Welt. 14: 507-508 (1940).

(7) Chediak, A.: Simplification of the serodiagnosis of syphilis. Havana, Cuba, Ministry of National Defense, "Chediak" Institute, 1948.

(8) Hogan, R. B., and Busch, S.: Filter paper microscopic test for syphilis, or the FPM test. A preliminary report. J. Ven Dis. Inform. 31: 37-45 (1950).

(9) Harris, Ad, and Olansky, Sidney: A study of the filter paper microscopic (FPM) test for syphilis. Preliminary report. J. Ven Dis. Inform. 32: 1-4 (1951).

(10) U. S. Public Health Service: Manual of serologic tests for syphilis. Supplement No. 22, J. Ven. Dis. Inform, 1947.


# A Micromodification Of the VDRL Slide Test


By GEORGE R. CANNEFAX, B.S.
HAROLD R. BEYER
EDGAR B. JOHNWICK, M.D.


Except for the lack of an authoritative sensitivity and specificity evaluation, the micromodification of the VDRL slide test apparently provides a relatively simple and satisfactory method of collecting and testing small amounts of blood from infants, young children, and adults who present a problem in the collection of blood by venipuncture.

The results of testing 1,388 simultaneously collected specimens by the regular and the micro-VDRL test techniques are presented in this report. A request has been made that this modification of the VDRL slide test be included in the next National Evaluation of Serodiagnostic Tests for Syphilis.

## Materials

*Melting point capillary tubes (Kimble Glass Co. item 34500):* Outside diameter, 1.5 to 2.0 mm.; length, 100 mm.; open at both ends. One hundred pieces are supplied in a corked glass vial. Prior to use the tubes are washed with Orvis detergent, rinsed with tap water followed by distilled water, and dried in a hot-air sterilizer. These tubes are used for the collection of blood specimens.

*Glass tubing (Kimble Glass Co. item 46470):* Glass tubing with an outside diameter of 4 mm., purchased in 4-foot lengths, and cut into 105-mm. lengths. The ends should be fire-polished. These tubes, when fitted with a rubber cap on each end, serve as carrying or protecting containers for the collection tubes.

*Rubber caps for closing both ends of the protection tube:* Micro rubber policemen as used with A. H. Thomas item 8804 or any cap that will fit 4-mm. glass tubing.

*Ungraduated micropipettes:* Drawn from 4-mm. outside diameter glass tubing. The pipette should have a


*Mr. Cannefax is laboratory director, Mr. Beyer, medical technician, and Dr. Johnwick, medical officer in charge, Public Health Service Medical Center, Hot Springs, Ark.*